"Express Mail" Label No. EL 823671200 US
Date of Deposit January 22, 2002

PATENT
Attorney Docket No.: 020167-000130US

I hereby certify that this is being deposited with the United States Postal Service "Express Mail Post Office to Address" service under 37 CFR 1.10 on the date indicated above and is addressed to:

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

By: Masha M. Martinenko
Masha M. Martinenko

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of:

Zhong-Ru Gan

Application No.: Not yet assigned
Continuation of Application No.: 09/423,100

Filed: Herewith

For: CHIMERIC PROTEIN CONTAINING AN INTRAMOLECULAR CHAPERONE-LIKE SEQUENCE

Art Unit: Not yet assigned

Examiner: Not yet assigned

PRELIMINARY AMENDMENT

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-referenced application, please enter the following amendments and remarks.

AMENDMENTS

IN THE TITLE:

Please amend the title to read as follows:

CHIMERIC PROTEIN CONTAINING AN INTRAMOLECULAR CHAPERONE-LIKE SEQUENCE

IN THE SPECIFICATION:

Please insert the following at the beginning of the specification:

Cross References to Related Applications

This application is a continuation application claiming benefit under 35 U.S.C. §120 of co-pending U.S. Patent Application No. 09/423,100 filed on December 11, 2000 which is the National Stage of International Application No. PCT/CN98/00052 filed on March 31, 1998, which was published in English under PCT article 21(2), the content of which are herein incorporated by reference in their entirety.

IN THE CLAIMS:

Please cancel claims 1-77.

Please enter the following new claims:

--78. A chimeric protein comprising:

a first peptidyl fragment;

a second peptidyl fragment comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment; and

at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;

the first peptidyl fragment having sufficient homology to at least a first 20 N-terminal amino acids of human growth hormone (hGH) protein that the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment.

79. A chimeric protein according to claim 78 wherein the C-terminus of the first peptidyl fragment is adjacent the N-terminus of the second peptidyl fragment.

80. A chimeric protein according to claim 78 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

81. A chimeric protein according to claim 78 wherein the first peptidyl fragment comprises SEQ ID NO:1.

82. A chimeric protein according to claim 78 wherein the first peptidyl fragment comprises SEQ ID NO:2.

83. A chimeric protein according to claim 78 wherein the first peptidyl fragment comprises SEQ ID NO:3.

84. A chimeric protein according to claim 78 wherein the first peptidyl fragment is between 20 and 200 residues in length.

85. A chimeric protein according to claim 78 wherein the second peptidyl fragment exhibits insulin-like bioactivity in its bioactive conformation.

86. A chimeric protein according to claim 78 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

87. A chimeric protein according to claim 78 wherein the second peptidyl fragment is an insulin precursor.

88. A chimeric protein according to claim 78 wherein the second peptidyl fragment is an insulin precursor of human origin.

89. A chimeric protein according to claim 78 wherein the second peptidyl fragment comprises SEQ ID NO:4.

90. A chimeric protein according to claim 78 wherein the second peptidyl fragment comprises SEQ ID NO:5.

91. A chimeric protein according to claim 78 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

92. A chimeric protein according to claim 78 wherein the second peptidyl fragment comprises at least four cysteine residues which form two cysteine bridges.

93. A chimeric protein according to claim 78 wherein the second peptidyl fragment comprises at least six cysteine residues which form three cysteine bridges.

94. A chimeric protein according to claim 78 wherein the cleavable peptidyl fragment is an Arg or Lys residue.

95. A chimeric protein according to claim 78 wherein the cleavable peptidyl fragment is at least 2 amino acids in length where the C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

96. A chimeric protein according to claim 78 wherein the protein comprises SEQ ID NO:6.

97. A chimeric protein according to claim 78 wherein the protein comprises SEQ ID NO:7.

98. A nucleic acid sequence encoding a chimeric protein, the chimeric protein comprising:

a first peptidyl fragment;

a second peptidyl fragment comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment; and

at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;

the first peptidyl fragment having sufficient homology to at least a first 20 N-terminal amino acids of human growth hormone (hGH) protein that the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment.

99. A nucleic acid sequence according to claim 98 wherein the C-terminus of the first peptidyl fragment is adjacent the N-terminus of the second peptidyl fragment.

100. A nucleic acid sequence according to claim 98 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

101. A nucleic acid sequence according to claim 98 wherein the first peptidyl fragment comprises SEQ ID NO:1.

102. A nucleic acid sequence according to claim 98 wherein the first peptidyl fragment comprises SEQ ID NO:2.

103. A nucleic acid sequence according to claim 98 wherein the first peptidyl fragment comprises SEQ ID NO:3.

104. A nucleic acid sequence according to claim 98 wherein the first peptidyl fragment is between 20 and 200 residues in length.

105. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment exhibits insulin-like bioactivity in its bioactive conformation.

106. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

107. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment is an insulin precursor.

108. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment is an insulin precursor of human origin.

109. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment comprises SEQ ID NO:4.

110. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment comprises SEQ ID NO:5.

111. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

112. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment comprises at least four cysteine residues which form two cysteine bridges.

113. A nucleic acid sequence according to claim 98 wherein the second peptidyl fragment comprises at least six cysteine residues which form three cysteine bridges.

114. A nucleic acid sequence according to claim 98 wherein the cleavable peptidyl fragment is an Arg or Lys residue.

115. A nucleic acid sequence according to claim 98 wherein the cleavable peptidyl fragment is at least 2 amino acids in length where the C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

116. A nucleic acid sequence according to claim 98 wherein the protein comprises SEQ ID NO:6.

117. A nucleic acid sequence according to claim 98 wherein the protein comprises SEQ ID NO:7.

118. A cell capable of expressing a recombinant protein which comprises:

a first peptidyl fragment;

a second peptidyl fragment comprising an amino acid sequence which comprises at least two cysteine residues which form at least one cysteine bridge in a bioactive conformation of the second peptidyl fragment; and

at least one cleavable peptidyl fragment linking the first and second peptidyl fragments;

the first peptidyl fragment having sufficient homology to at least a first 20 N-terminal amino acids of human growth hormone (hGH) protein that the first peptidyl fragment mediates formation of the bioactive conformation of the second peptidyl fragment.

119. A cell according to claim 118 wherein the C-terminus of the first peptidyl fragment is adjacent the N-terminus of the second peptidyl fragment.

120. A cell according to claim 118 wherein the first peptidyl fragment is capable of being bound by an anti-hGH antibody.

121. A cell according to claim 118 wherein the first peptidyl fragment comprises SEQ ID NO:1.

122. A cell according to claim 118 wherein the first peptidyl fragment comprises SEQ ID NO:2.

123. A cell according to claim 118 wherein the first peptidyl fragment comprises SEQ ID NO:3.

124. A cell according to claim 118 wherein the first peptidyl fragment is between 20 and 200 residues in length.

125. A cell according to claim 118 wherein the second peptidyl fragment exhibits insulin-like bioactivity in its bioactive conformation.

126. A cell according to claim 118 wherein the second peptidyl fragment is capable of being bound by an anti-human-insulin antibody.

127. A cell according to claim 118 wherein the second peptidyl fragment is an insulin precursor.

128. A cell according to claim 118 wherein the second peptidyl fragment is an insulin precursor of human origin.

129. A cell according to claim 118 wherein the second peptidyl fragment comprises SEQ ID NO:4.

130. A cell according to claim 118 wherein the second peptidyl fragment comprises SEQ ID NO:5.

131. A cell according to claim 118 wherein the second peptidyl fragment comprises A chain and B chain amino acid sequences of human insulin separated by an amino acid sequence between 1 and 34 residues in length.

132. A cell according to claim 118 wherein the second peptidyl fragment comprises at least four cysteine residues which form two cysteine bridges.

133. A cell according to claim 118 wherein the second peptidyl fragment comprises at least six cysteine residues which form three cysteine bridges.

134. A cell according to claim 118 wherein the cleavable peptidyl fragment is an Arg or Lys residue.

135. A cell according to claim 118 wherein the cleavable peptidyl fragment is at least 2 amino acids in length where the C-terminal amino acid residue is selected from the group consisting of Arg and Lys.

136. A cell according to claim 118 wherein the protein comprises SEQ ID No:6.

137. A cell according to claim 118 wherein the protein comprises SEQ ID No:7.--

IN THE ABSTRACT:

Please replace the Abstract with the following:

The present invention relates to a chimeric protein containing an intramolecular chaperone (IMC) like sequence linked to a target protein including, but not limited to, an insulin precursor protein. The present invention also relates to polynucleotides encoding such proteins and cells capable of expressing such proteins. The present invention also relates to compositions for obtaining a correctly folded insulin-precursor-containing chimeric protein, comprising, *inter alia*, contacting an incorrectly folded chimeric protein containing an IMC like sequence linked to an insulin precursor with at least one chaotropic auxiliary agent. The present invention also relates to compositions for screening polypeptide amino acid sequences for an ability to improve folding of an insulin precursor using a chimeric protein containing an IMC like sequence linked to an insulin precursor. The present invention provides compositions for obtaining correctly folded recombinant proteins.

REMARKS

The above amendments add no new matter.

## CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 925-472-5000.

Respectfully submitted,

Frank J. Mycroft
Reg. No. 46,946

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: (925) 472-5000
Fax: (925) 472-8895
FJM:mmm
WC 9036091 v1